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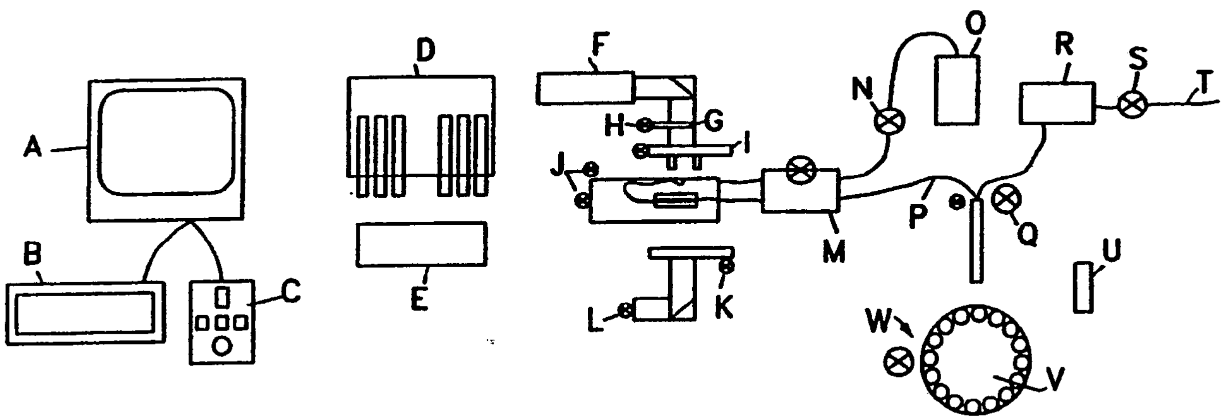
(54) Title: METHOD AND APPARATUS FOR PREPARING A LIQUID SPECIMEN



(57) Abstract

A method and apparatus are provided for accurately preparing centrifuged fluid samples for microscopic analysis. In the method a centrifuged fluid specimen contained in a sample tube of known volume and dimensions located in motor (W) driven sample tube cassette carousel (V) has the level of fluid specimen in the sample tube detected therein with a sensing duel pipette tube (D6). This level is used in calculating the total volume of fluid specimen in the sample tube with the computer (D). Thereafter, an amount of fluid specimen sufficient to leave a predetermined volume percentage of fluid specimen in the sample tube is decanted. Next the sample tube is agitated to resuspend the sediment that had been concentrated in the centrifuged fluid speci-

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METHOD AND APPARATUS FOR PREPARING A LIQUID SPECIMEN

BACKGROUND OF THE INVENTION

The present invention relates generally to the field of automated instrumentation, and more specifically to the field of microscopic analysis.

One known system for microscopically analyzing fluids is disclosed in United States Letters Patent No. 4,804,267 to Greenfield, assigned to the assignee of the present invention, the disclosure of which is incorporated herein by reference. Although the Greenfield system provided effective solutions to many of the problems confronting the art it itself possesses several disadvantages and drawbacks.

Specifically, the system of the '267 patent utilizes a single pump and flushes the specimen through the system to waste. Experience has shown that this may allow for the introduction of bubbles into the flow cell which can be seen as artifacts under high magnification. In addition, the system of the '267 patent provides only a limited numb r of user functions or features.

There is a long felt need, which has gone unsatisfied prior to the making of the present invention, for an automated system for microscopically analyzing fluids,

which provides rapid, accurate, multiple sample viewing capability, and the ability to control the viewing and analysis of the specimen including saving it, if necessary. Also there exists an unsatisfied, long felt need for a method and means for accurately preparing a fluid specimen for microscopic analysis.

It is accordingly a general object of the present invention to overcome the aforementioned limitations and drawbacks associated with known systems and to fulfill the needs mentioned above by providing a system for microscopically analyzing a specimen having all of the desirable attributes noted above.

It is a particular object of the present invention to provide a method for accurately preparing fluid specimens for microscopic analysis.

It is another object of the present invention to provide an automated method for preparing fluid specimens for microscopic analysis.

Another object of the present invention is to provide a method for standardizing the preparation of fluid specimens for microscopic analysis.

A further object of the present invention is to provide a means of mixing (resuspending) the fluid sediment.

The foregoing and other objects and advantages which will be apparent in the following detailed description of the preferred embodiment, or in the practice of the invention, are achieved by the invention disclosed herein, which generally may be characterized as a method for preparing for microscopic analysis a centrifuged fluid specimen contained in a sample tube of known volume and dimensions comprising the steps of:

detecting the level of fluid specimen in the sample tube; calculating the total volume of fluid specimen in the sample tube; decanting an amount of fluid specimen to leave a predetermined volume percentage of fluid specimen remaining in the sample tube; and agitating the predetermined percentage of fluid specimen remaining in the sample tube to resuspend the sediment that had been concentrated in the centrifuged fluid specimen, and apparatus for preparing for microscopic analysis a centrifuged fluid specimen contained in a sample tube of known volume and dimensions comprising: means for detecting the level of fluid specimen in the sample tube; means for calculating the total volume of fluid specimen in the sample tube; means for decanting an amount of fluid specimen to leave a predetermined volume percentage of fluid specimen in a sample tube; and means for agitating the predetermined percentage of fluid specimen remaining in the sample tube to resuspend the sediment that had been concentrated in the centrifuged fluid specimen.

BRIEF DESCRIPTION OF THE DRAWINGS

Serving to illustrate exemplary embodiments of the invention are the drawings, of which:

Figure I is a block diagram of the robotic microscope of the present invention;

Figure 2 is a diagram illustrating the internal components of the three subsystems of the robotic microscope of the present invention;

Figure 3 is an exploded diagram of a multi-channel flow cell in accordance with the present invention;

Figure 4 is block diagram of a multi-channel flow cell with pumping/sampling system in accordance with the present invention;

Figure 5 is a block diagram of a multi-channel pipette and pumping/sampling system in accordance with the present invention; and

Figure 6 is a block diagram of apparatus for preparing a specimen in accordance with the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

As used herein, and in accordance with the present invention, a robotic microscope is a computerized optical imaging instrument which automates various aspects of microscopic laboratory tests and is designed to assist the trained laboratory technician in performing routine microscopic analysis, such as, for example, urinalysis. Among the benefits over conventional approaches are improved accuracy and standardization; improved ability to visualize difficult specimens; reduced specimen handling; reduced disposables; and higher productivity for the laboratory.

Referring to Figure 1, a diagram of the three subsystems of the robotic microscope of the present invention is illustrated. As shown therein, the robotic microscope consists of a Control Station, an Imaging Station and a Specimen Station.

Referring now to Figure 2, the internal components of the three subsystems of the robotic microscope are illustrated. As shown therein, the Control Station includes a monitor for viewing the microscopic specimen, a keyboard and other computer input device (such as a mouse or trackball) as necessary to interface with the

computer controls of the microscope. The Control Station is where the laboratory technologist controls the microscope, performs the analysis and records the results.

The screen on the monitor displays a highly magnified image of the specimen as well as relevant data about the specimen and alphanumeric and graphical symbols which, when used in conjunction with the proper input device, allow a technician to control all the parameters required for the microscopic examination. In particular, while the magnified specimen is being viewed, the technician can also control the following functions: magnification; focus; location; scanning; sampling; optical enhancement; lighting; and sample data entry.

The Imaging Station includes a high magnification optical and video system, a computer, input/output interface, power supply, filters, flow cell, apertures, necessary motors and control electronics. The Imaging Station is where a magnified electronic image of the specimen to be analyzed is obtained.

The Specimen Station includes apparatus to deliver the specimen to the viewing area of the Imaging Station and means for removing the specimen from the Imaging Station after it has been analyzed. In the instance of solid or dried specimen on a slide, the Specimen Station consists of a mechanical device to load and unload slides. In the instance where the specimen is wet or in liquid state, the Specimen Station includes a pumping system to pump specimen into and flush specimen from the viewing area of the Imaging Station and an indexing system to index sequential samples.

The Specimen Station also includes a carousel which holds the specimen cassette tray. Each cassette holds up to 20 specimens in sample tubes and is coded and numbered to assure accurate transcription of patient information. Directly behind the cassette is the washbowl, and above both is the sampling autopipette. To the left of the carousel and autopipette is the flush reservoir, which is accessed via the top cover door.

The majority of all operations performed on the robotic microscope are done with the Trackball and the corresponding cursor on the Main Control Screen.

The operation of the robotic microscope illustrated in Figures 1 and 2 is as follows. After turning on the power switch and waiting for instrument to complete its automatic priming and self-testing functions, the technician loads the sample tubes into the Sample Tube Cassette (V). The technician then uses the trackball (C) to activate the computer controls on the monitor screen (A) and instructs the instrument to prepare the next sample.

The instrument then indexes the Carousel Motor (W) until Sample Tube Cassette (V) has turned a sample tube into position under the Two Axis motorized Pipette (Q). The pipette (Q) then decants the specimen and pumps it via tubing (P), valves (M) through the flow cell (J) via the sampling pump (N).

The technician then performs the analysis of the specimen, viewing it on screen (A) via computer (D), Camera (F), Optical Column (G), Lens (I), flow cell (J), Apertures (K), and Illuminator (L).

After completing the analysis, the technician enters the report via keyboard (B), and the system is automatically

flushed via sample pump (N), Flush Reservoir (O), out to washbowl (U). The waste pump (S) then takes the specimen and flush solution from the washbowl (U) through the pipette (Q) out through the waste port (T). The instrument is now ready for the next sample.

All of these functions are controlled by the technician through the computer (D) and powered by the power supply (E).

In accordance with the present invention, multiple sample viewing capability for liquid samples is provided by means of a multi-channel flow cell. Although the following discussion of the multi-channel flow cell is in terms of a dual channel flow cell, it is clear that the number of channels can be increased accordingly.

The general operation of the dual channel flow cell is as follows. The specimen is first pumped into the alpha channel of the flow cell. While the operator is examining the specimen under optical magnification on the monitor screen the pump flushes and then loads the beta channel of the flow cell. When the operator has completed due examination of the specimen contained in the alpha channel, the Imaging Station moves the beta channel of the flow cell into view under the optical system. This provides the operator with extremely rapid access to sequential prepared samples because the time required to prepare the second sample is coincident with the operator's time to view the previous sample, thus there is no waiting time for the operator while the sample is prepared.

Referring to Figures 3 and 4, an exploded diagram of a dual channel flow cell, and a dual channel flow cell with pumping/sampling system, respectively, are

cell requires an input line and an output line for both alpha and beta channels and a valve system on each end to switch the pump between channels. The valves also guarantee that the specimen will be held rigidly in place during microscopic examination.

The dual channel flow cell consists of two sample channels or chambers the depth of which are determined by the working distance and top cover thickness given the optical characteristics of the objectives. The thickness of the top cover is also determined by the objective while the thickness of the bottom is determined by the working distance of the condenser lens.

The dual channel flow cell includes transparent upper and lower retaining members, generally flat in form, one of which has a plurality of pairs of fluid flow passages formed therein. It also includes a central member, generally flat in form, having a plurality of display chambers defined therein. Each of the display chambers is in fluid flow registration with one of the pairs of fluid flow passages and each of the display chambers is out of fluid flow registration with all of the other of the plurality of display chambers. The upper, lower and central members are secured to one another to form an integral structure, which is carried in a body having a flat central well and a viewing aperture formed therein. At least a portion of each of the plurality of display chambers underlies the viewing aperture.

The operation of the multi-channel flow cell is as follows. (1) The sample is taken up in pipette D4 through the first channel of the two channel pipette via the action of sample pump E4. (2) After the sample has passed through valve C4 and into the alpha channel of the Flow cell A4, the action of pump E4 ceases and then

the valve C4 switches to the other channel (beta). (3) The pipette is then inserted into the next sample and steps 1 and 2 are repeated with the exception that the sample is pumped into the beta channel and valve C4 subsequently switches to channel alpha. (4) To remove the sample, the pipette is inserted into the washbowl (not shown). (5) The sample pump is then operated in reverse mode and pumps flush solution out of flush solution reservoir G4 through the alpha channel of flow cell A4, through valve C4 and through first channel of pipette D4, thereby pushing sample into washbowl and filling alpha channel of the flow cell with flush solution. (6) The waste pump F4 is then activated and pulls specimen and flush solution out of the washbowl up through the second channel of pipette D4 and out through waste port H4. (7) Steps 4 through 6 are repeated to clean out the beta channel of the flow cell with the exception that the valve C4 is first set to the beta channel. (8) The same procedures as listed above can also be used for more than two channel flow cells, requiring only more channels in the flow cell itself and greater switching capacity in the valve.

In accordance with the present invention, a means of sampling liquid specimens such that each sample channel of a multi-channel pipette and its associated tubing acts as a reservoir of specimen is provided.

Referring to Figure 5, a multi-channel pipette and pumping/sampling system in accordance with the present invention is illustrated. As shown therein, the specimen is sampled via a pipette which descends into the sample tube. The pipette consists of three rigid tubes or channels, two for sample and an auxiliary one for waste. Each sample channel in the pipette corresponds to one of the channels in the flow cell.

rigid tubes of the pipette to the valves, and then from the valves to the flow cell.

The volume of specimen contained in the rigid and flexible tubing functions as a reservoir, holding additional sample. This allows more sample to be viewed in addition to the sample that is in the viewing channel of the flow cell. Further, this reservoir of specimen in the tubing allows the sample to be saved in its entirety as opposed to being lost in the flushing process. These design advantages are juxtaposed to a system that has only a single tube for taking up the sample. Such a system would require a valve to switch between the two channels of the flow cell. If the advantages of speed from the two channels of the flow cell were to be maintained, then the balance of the specimen would either be left in the sample tube (requiring a separate operation to extract it if additional specimens were to be viewed) or the specimen would be lost.

The multi-channel pipette system, described in the system shown in Figure 5 has all of the same functional capabilities as the multi-channel flow cell system of Figure 4, but provides additional capabilities as well. In particular, by dedicating one channel of the pipette directly to one channel of the flow cell, the tubing can function as a reservoir of additional sample thereby providing additional system features such as sample advance; sample saving; sample staining all of which require the sample reservoir to be functional.

The operation of the multi-channel pipette is as follows. The system functions the same as the system described in Figure 4 above with the exception that a separate sample channel in the pipette and its associated tubing correspond to one of the sample-

channels in the multi-channel flow cell. In addition, it has the following operations. (1) To advance the sample into the flow cell, the valve C5 is switched to the appropriate channel of the flow cell being viewed. (2) The sample pump is then run in the direction to pull the sample towards the pump by an amount equal to the volume of the channel of the flow cell. (3) The valve is then switched back to the other channel. (4) Sample saving is similar to the flushing operation described in the system of Figure 4, with the exception that the pipette is placed back in the original sample tube instead of the washbowl, and the waste pump is never activated. (5) Sample staining is the same as sample saving with the exception that after the sample has been saved, the technician then adds stain to the sample and then it is resampled to the flow cell in the procedure described in Figure 4 above.

In accordance with the present invention, the system also provides for the capability to rapidly and accurately prepare a suspension of sample from a centrifuged fluid specimen, such as a urine specimen. After the specimen is centrifuged, the biological sediment is concentrated in the bottom of the sample tube. The dual or multi-channel pipette then descends into the sample tube, and, utilizing a sample channel and the auxiliary (waste) channel in the pipette as sensor probes, detects the level of the fluid in the sample tube and determines the amount of fluid in the sample tube. The two channels consist of rigid metal tubing electrically isolated from each other within the pipette and are used with known circuitry to detect the changes in conductivity between the air and the fluid in the sample tub . The computer then calculates the total volume of fluid in the sample tube and determines the amount of fluid to be decanted in order to leave a

tube. The pipette then descends to this predetermined level and decants the fluid through the auxiliary channel in the pipette along the way. The pipette then descends further into the sample tube and the auxiliary channel in the pipette and the waste pump then cycle vigorously. This back and forth cycling of the pump agitates the button of biological sediment in the bottom of the sample tube creating a concentrated suspension. This system allows for an exact suspension to be prepared independently of the initial volume presented. In practice, the current manual approaches are extremely lax in the precision with which the concentration is prepared.

Referring to Figure 6, a method and apparatus for accurately preparing liquid samples that have centrifuged sediment in them in accordance with the present invention is illustrated. Although a multi-channel flow cell is shown therein, it is noted that the system does not require the use of a multi-channel flow cell.

Its operation is as follows: (1) The pipette D6 descends into the sample tube (not shown) and detects the level of sample liquid. (2) The system calculates the volume and determines the amount to be decanted to create a proportional specimen (i.e. a 10% suspension of sediment and original sample supernatant). The system decants this proportion via Valve F6 and pump G6. (3) The system then cycles via valve F6 and Pump G6 (that is pumps rapidly and forcefully backwards and forwards) with the pipette immersed in the sample. This breaks up centrifuged constituents and causes them to create a suspension of said constituents that is highly and proportionally concentrated in comparison to the original liquid volume. (4) The specimen is then

sampled as in a procedure described for either system in Figure 4 or 5.

Although the present invention has been described in terms of its presently preferred embodiment, certain modifications thereof based on the descriptions and teachings herein may be apparent to those skilled in the art. For example, the embodiment disclosed above deals with a system for microscopically analyzing fluids. An adaptation of the invention to other forms of specimens such as solid samples should be apparent to those skilled in the art.

Accordingly, the scope of the present invention is defined by the following claims.

WHAT IS CLAIMED IS:

1. A method for preparing for microscopic analysis a centrifuged fluid specimen contained in a sample tube of known volume and dimensions comprising the steps of:

   detecting the level of fluid specimen in the sample tube;

   calculating the total volume of fluid specimen in the sample tube;

   decanting an amount of fluid specimen to leave a predetermined volume percentage of fluid specimen in the sample tube; and

   agitating the predetermined percentage of fluid specimen remaining in the sample tube to resuspend the sediment that had been concentrated in the centrifuged fluid specimen.

2. A method as recited in Claim 1 wherein the steps thereof are automated.

3. Apparatus for preparing for microscopic analysis a centrifuged fluid specimen contained in a sample tube of known volume and dimensions comprising:

   means for detecting the level of fluid specimen in the sample tube;

   means for calculating the total volume of fluid specimen in the sample tube;

   means for decanting an amount of fluid specimen to leave a predetermined percentage volume of fluid

specimen in the sample tube; and

means for agitating the predetermined percentage of the fluid specimen remaining in the sample tube to resuspend the sediment that had been concentrated in the centrifuged fluid specimen.

4. Apparatus as recited in Claim 3 wherein said detecting means, said calculating means, said decanting means and said agitating means are controlled by a computer.

5. Apparatus as recited in Claim 4 wherein said detecting means includes pipette means configured to detect changes in conductivity between the air and the fluid.

6. Apparatus as recited in Claim 5 wherein said calculating means includes computer means.

7. Apparatus as recited in Claim 6 wherein said decanting means include pipette means having at least an auxiliary channel, waste pump means and waste valve means, said auxiliary channel of said pipette means being connected to said waste pump means and waste valve means by fluid flow connection means.

8. Apparatus as recited in Claim 7 wherein said agitating means include said pipette means having at least an auxiliary channel, said waste pump means and said waste valve means, said auxiliary channel of said pipette means being connected to said waste pump means and said waste valve means by fluid flow connection means.

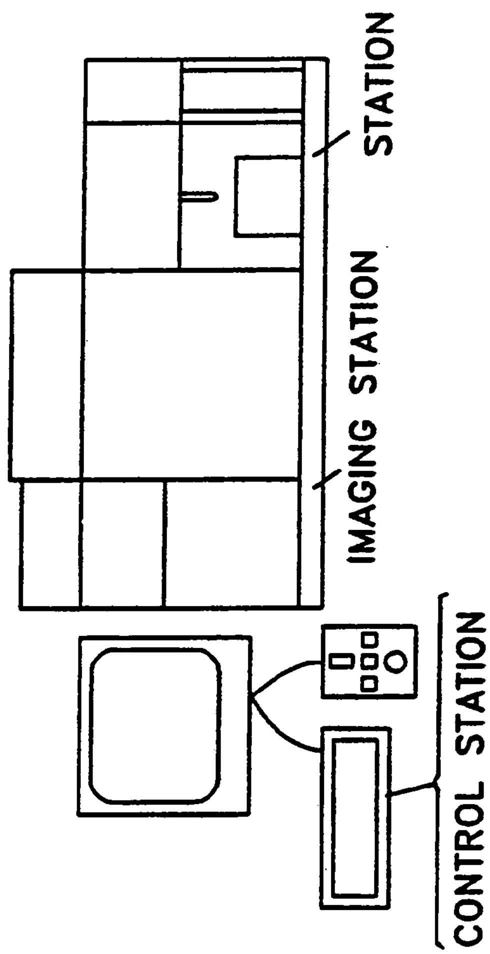


FIG. 1

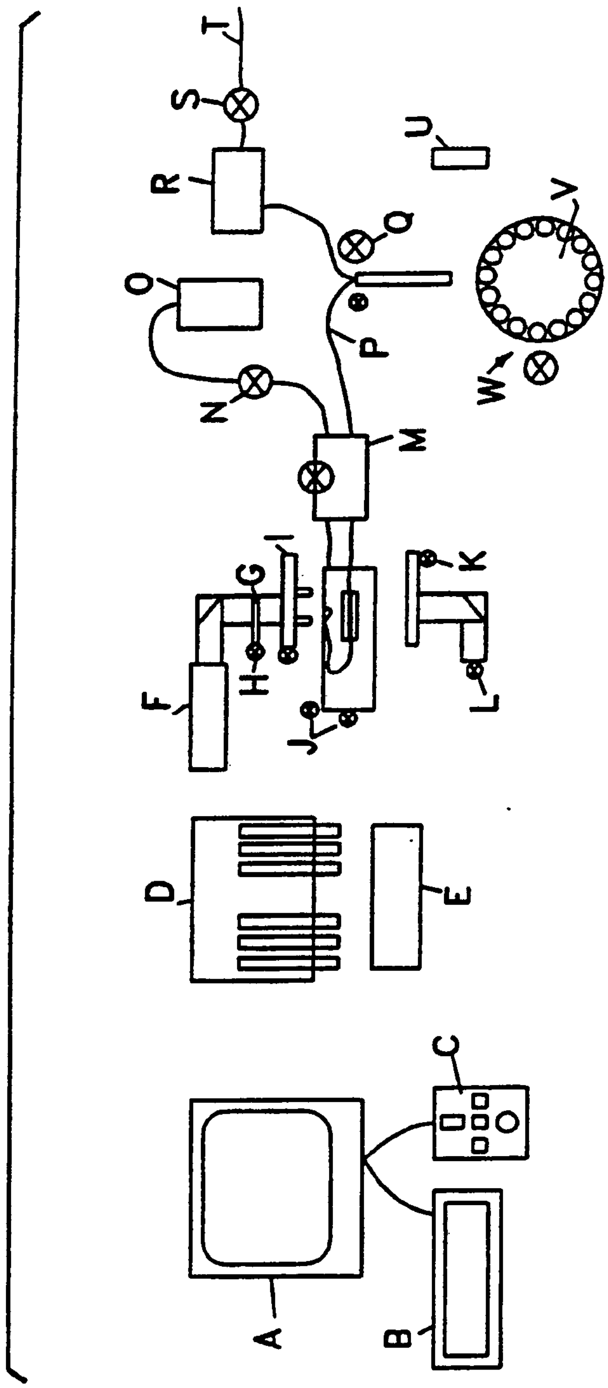


FIG. 2

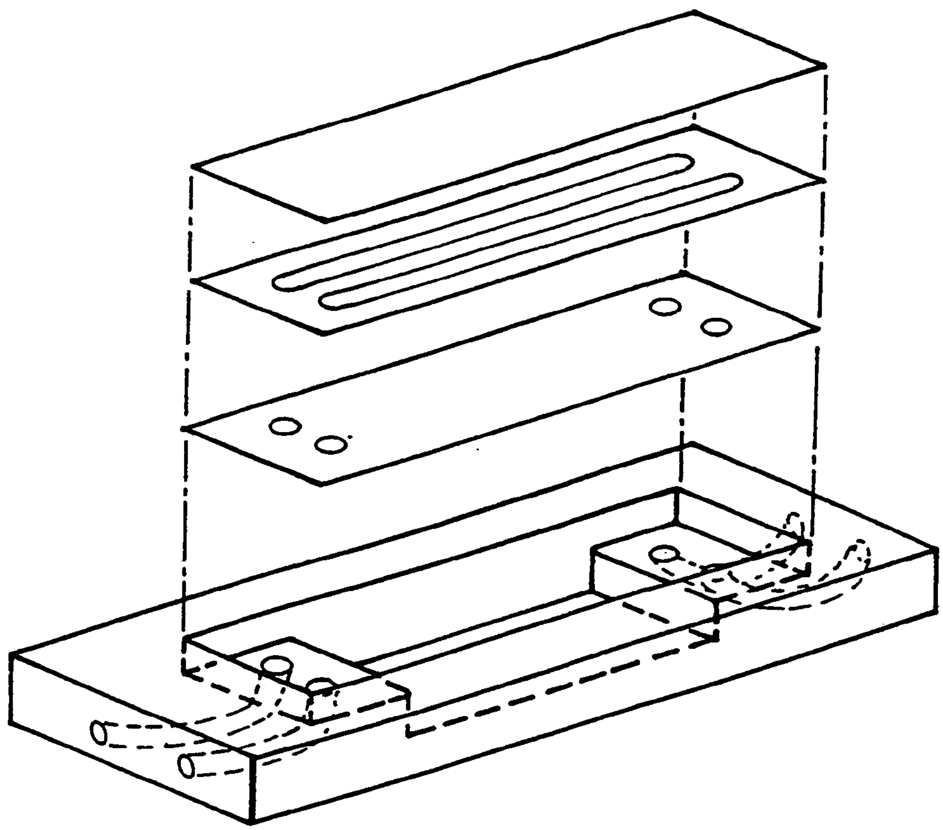
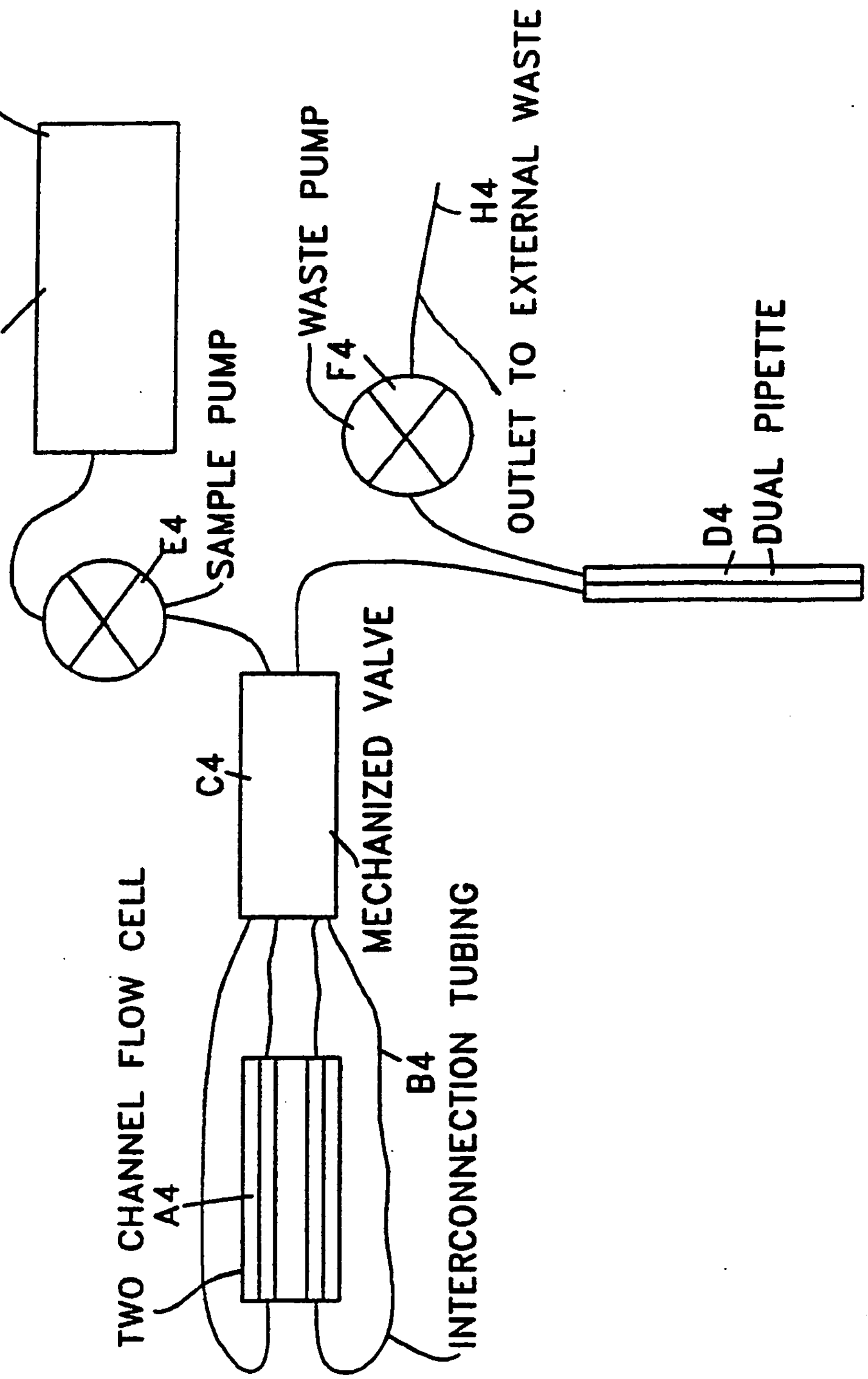


FIG. 3

FIG. 4



MULTI-CHANNEL FLOW CELL (TWO CHANNELS SHOWN) WITH PUMPING/SAMPLING SYSTEM

FIG. 5

MULTI - CHANNEL PIPETTE AND PUMPING SAMPLING SYSTEM

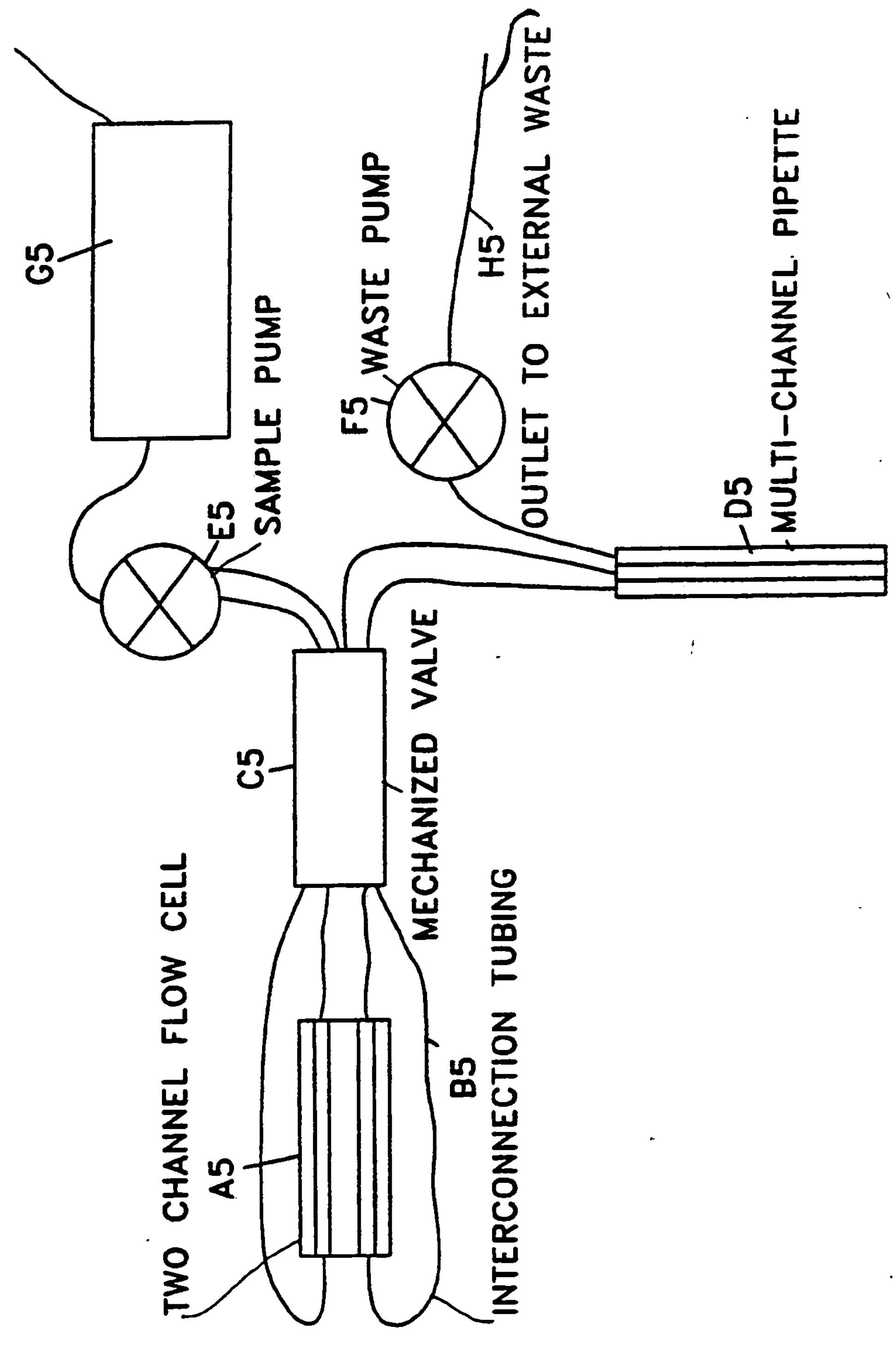
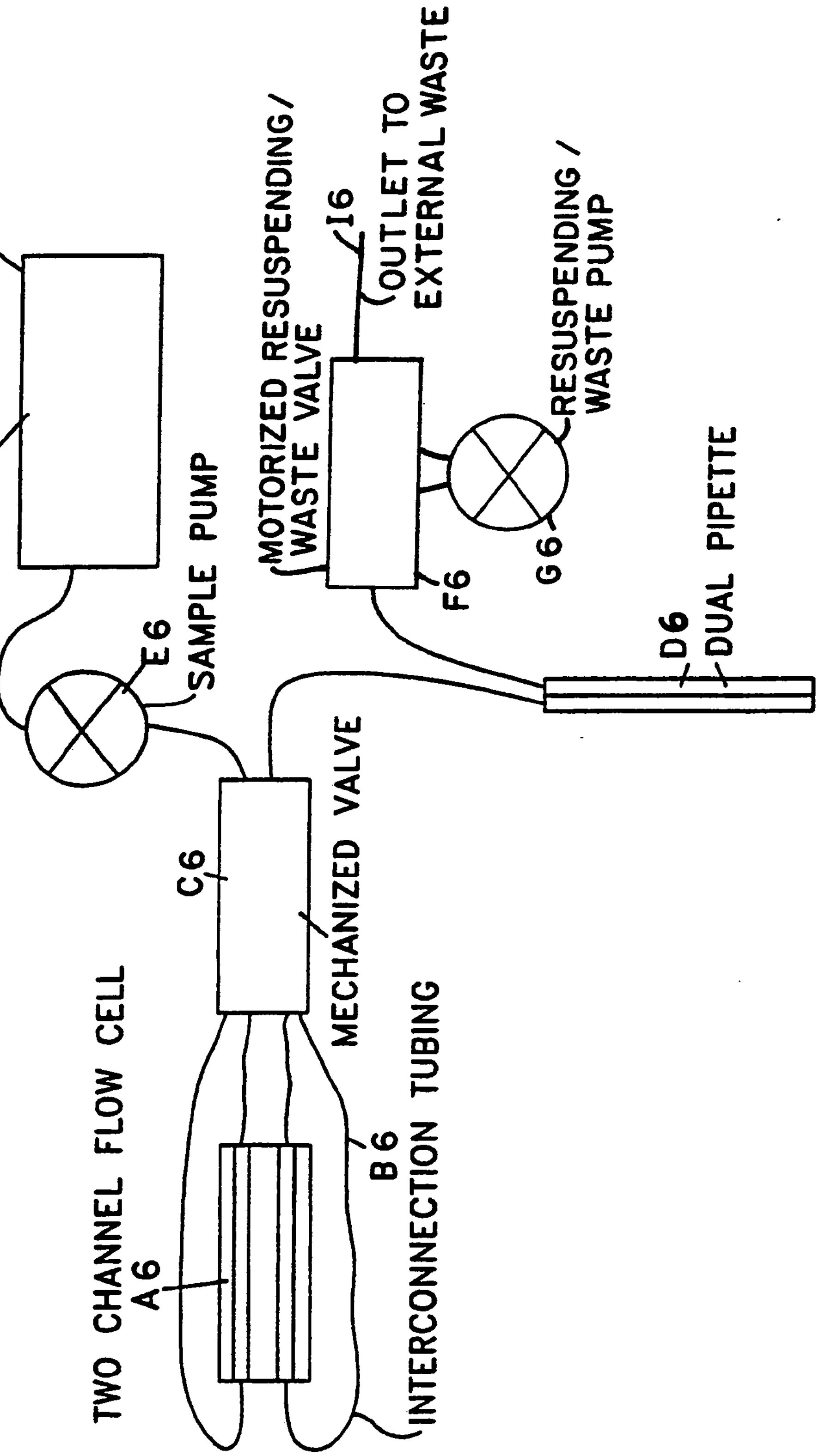


FIG. 6



METHOD AND MEANS FOR PREPARING SPECIMEN

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/03389

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

US CL: 73/863,863.21,863.32,863.02;356/36

IPC(5): G01N 1/28

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	73/863,863.21,863.32,863.01,863.02,864.11,864.15,864.21, 864.22; 356/36, 366/140; 436/45,174,177; 422/64

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

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Y	US, A, 4,435,293 (GRAHAM JR., ET AL.) 06 MARCH 1984 Note col. 1, lines 14-65 and col..9, line 56-col. 11, line 24.	1-6
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Y	US, A, 4,486,315 (TEIPEL) 04 DECEMBER 1984, Note col. 6, line 58-col. 7, line 5.	1-6
Y,P	US, A, 4,939,925 (SAKUMA ET AL.) 10 JULY 1990 Note abstract, Fig. 1, and Fig. 2.	1-6

* Special categories of cited documents: [10]

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IV. CERTIFICATI N

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18 MAY 1991	16 AUG 1991

International Searching Authority	Signature of Authorized Officer
	Andre Robinson

International Application No. PCT/US91/03389

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FR M THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
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